Current Research Journal of Biological Sciences 4(5): 584-591, 2012
ISSN: 2041-0778

Submitted: June 01, 2012 Accepted: June 21, 2012 Published: September 20, 2012

Species Richness and Spatial Distribution of Pathogenic *Vibrio* (Bacteria, *Vibrionaceae*) in Tropical Surface Waters: Yaoundé Metropolis Case (Cameroon, Central Africa)

[1]Tamatcho Kweyang Blandine Pulchérie, [1,2]Fotsing Kwetche Pierre René, [1]Nougang Mireille Ebiane, [1]Zébazé Togouet Serge Hubert and [1]Njiné Thomas
[1]Laboratory of General Biology, Hydrobiology and Environment Unit, Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé I, P.O. Box 812 Yaoundé, Cameroon
[2]Laboratory of Clinical Microbiology, Université des Montagnes, P.O. Box 208 Bangangté, Cameroon

Abstract: In the present study, six species of *Vibrio* that are potential human pathogens have been isolated from surface water resources in an urban environment and have been identified. The variation of some physico-chemical factors known to have significant effects on bacterial growth and spread are also considered. The latter include: electrical conductivity, pH, total suspended solids, temperature, dissolved oxygen and carbon dioxide, and rate of water flow. All investigations were conducted according to standard methods. Application of classical bacteriological techniques led to the identification of six species of the genus. *Vibrio cholera*, *Vibrio fluvialis*, *Vibrio mimicus*, *Vibrio alginolyticus*, *Vibrio parahaemolyticus* and *Vibrio vulnificus*. *Vibrio cholerae* was isolated in all water sampling points, with the highest frequency 79% upstream the Mfoundi River at Etoa-Meki. Rates of isolation of *Vibrio mimicus* increased with increased O_2 ($p<0.05$). Identification of these potential pathogens is a serious public health hazard highlighting the needs for resource management.

Keywords: Aquatic environment, Cameroon, physico-chemical factors, *Vibrio*, yaoundé

INTRODUCTION

Many infectious diseases that impact human and animal lives throughout the world are waterborne or water related (WHO, 2004). These diseases are more frequently reported in poor and developing countries as consequence of resource limitation and poor sanitation, generally associated with low purchasing power of the populations (Nguendo Yongsi *et al*., 2008; Seidu *et al*., 2008) and represent serious public health issues in those areas. Such diseases include bacterial diarrhea, bacterial enteritis, deep infections, cholera and dermatitis (Leclerc, 2003).

Clinically, cholera is characterized by excess lost of body fluid either through vomiting or rice watery-like diarrhea which may result in severe dehydration and subsequent death. Endemicity of cholera was reported in some developing countries since 1970 (Dosso *et al*., 1998) and the number of affected areas is on a permanent rise. The causative agent of cholera, *Vibrio cholerae (V. cholerae)*, belongs to the genus *Vibrio*, all species of which are natural hosts of watery environment (Fournier, 1998; Fournier and Quilici, 2002). Because *V. cholerae* is the species commonly incriminated in cholera outbreaks, it is also the most frequently targeted by almost all research initiatives. Strains from other species may play significant roles in human diseases but are frequently neglected (Duraku and Panariti, 1998).

Nowadays, in many settings, the fast population growth contrasts with shortage of safe drinking water because of large-scale pollution and global warming that are increasingly becoming issues of great concern. More and more, many populations around the world and specifically those from the areas where basic means for life are hardly provided use water from doubtful origins to meet their daily needs. Consequently, the number of people exposed to and affected by waterborne diseases also increases steadily (Nola *et al*., 1998; WHO, 2004). The populations of Douala, the economic capital city of Cameroon and those of Maroua, the headquarter of the Far North Region of the country, are parts of those experiencing recurrent outbreaks of cholera (Gévart *et al*., 2006), always associated with poor sanitation. Currently in Cameroon, cholera is documented in the headquarters of six regions out of ten. These include: Far North, North, Center, West, Littoral and South West regions (Fig. 1). Apart from *V. cholerae*, less is known about other potential disease-causing *Vibrio* species in Cameroon. Also, environmental factors that play major roles in recurrent outbreaks are not fully understood yet,

Corresponding Author: Tamatcho Kweyang Blandine Pulchérie, Laboratory of General Biology, Hydrobiology and Environment Unit, Department of Animal Biology and Physiology, Faculty of Science, University of Yaoundé I, P.O. Box 812 Yaoundé, Cameroon, Tel.: +23799784835

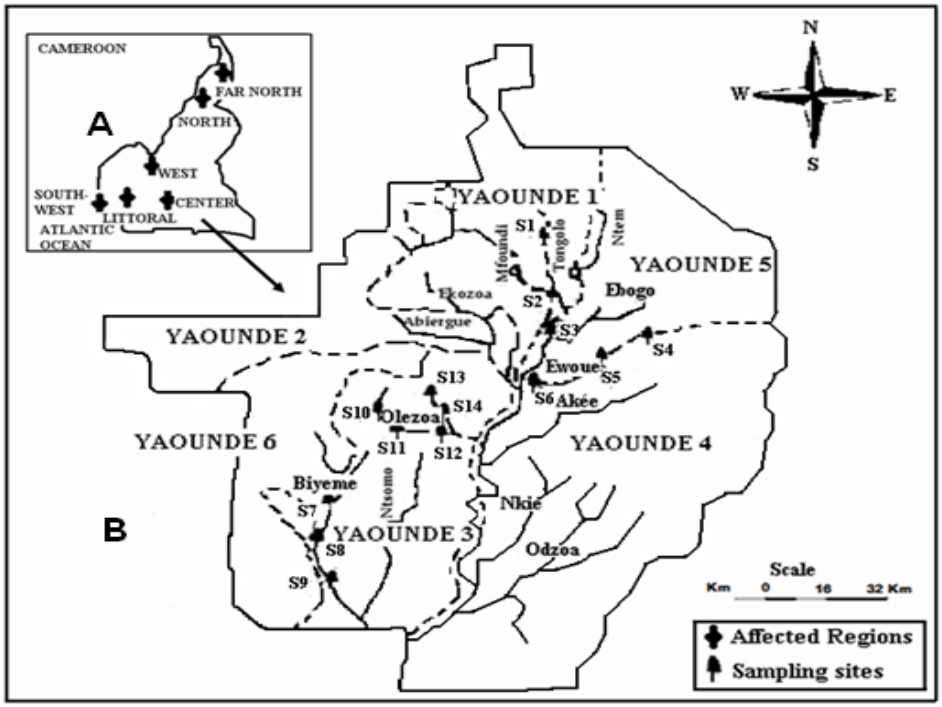

Fig. 1: (A) Spatial distribution of affected localities throughout the country, (B) Spatial distribution of sampling sites in Yaoundé

although saline sea water and soil porosity have long been recognized as contributors to *Vibrio* species growth and dissemination (Gévart *et al*., 2006). A good knowledge of these factors could help preventing future outbreaks, based on monitoring environmental changes, assessing exposure of populations to all *Vibrio* species through identification of bacteria in water resources.

If this is the first time an outbreak of cholera is documented in Yaoundé, previous surveys (Njiné *et al*., 2001; Nguendo Yongsi *et al*., 2008; Fotsing Kwetché *et al*., 2008, 2010; Tamatcho Kweyang *et al*., 2009, 2010) have consistently pointed out poor hygienic practices and stressed the need to improve the living conditions of Yaoundé city dwellers. This lack of hygiene prompted us to anticipate and investigate in a former survey through *Vibrio* species the Yaoundé populations are actually exposed to Tamatcho Kweyang *et al.* (2009). The present paper brings out the findings recorded in the continuation of that previous study. Isolation rates of different species were coupled with variations of some environmental physico-chemical factors. The results will serve in developing basic preventive guides against the current and future threats.

MATERIALS AND METHODS

Description of the study site: The present study was conducted on the streams of the Mfoundi hydrographic network in which domestic and industrial waste waters are poured. The choice of various sampling sites was made based on the use made of water by the local populations.

Sampling sites and samples analyses: A total of 14 surface water sites (numbered from S1 to S14) were concerned (Fig. 1). Water samples were collected twice a month from February (dry season) through July (rainy season). Physico-chemical and hydrological characteristics investigated included: temperature, pH, electrical conductivity, total suspended solids, dissolved oxygen, carbon dioxide and rate of water flow.

In the physico-chemical study, 1000 mL polyethylene made bottles were used to collect water samples that were transported at ambient temperature to the laboratory for analyses.

The pH and electrical conductivity were measured *in situ* with a SCHOTT GERÄTE pH-meter CG 818 and a HANNA conductimeter HI 8733, respectively. For dissolved oxygen and carbon dioxide, they were fixed *in situ*. The concentration determination was later performed in the laboratory by using respectively Winklers and hydrochloric acid the methods (Clesceri and Franson, 1998).

A spectrophotometer (HACH DR/2000) and a mercury assisted thermometer were used, to assess the

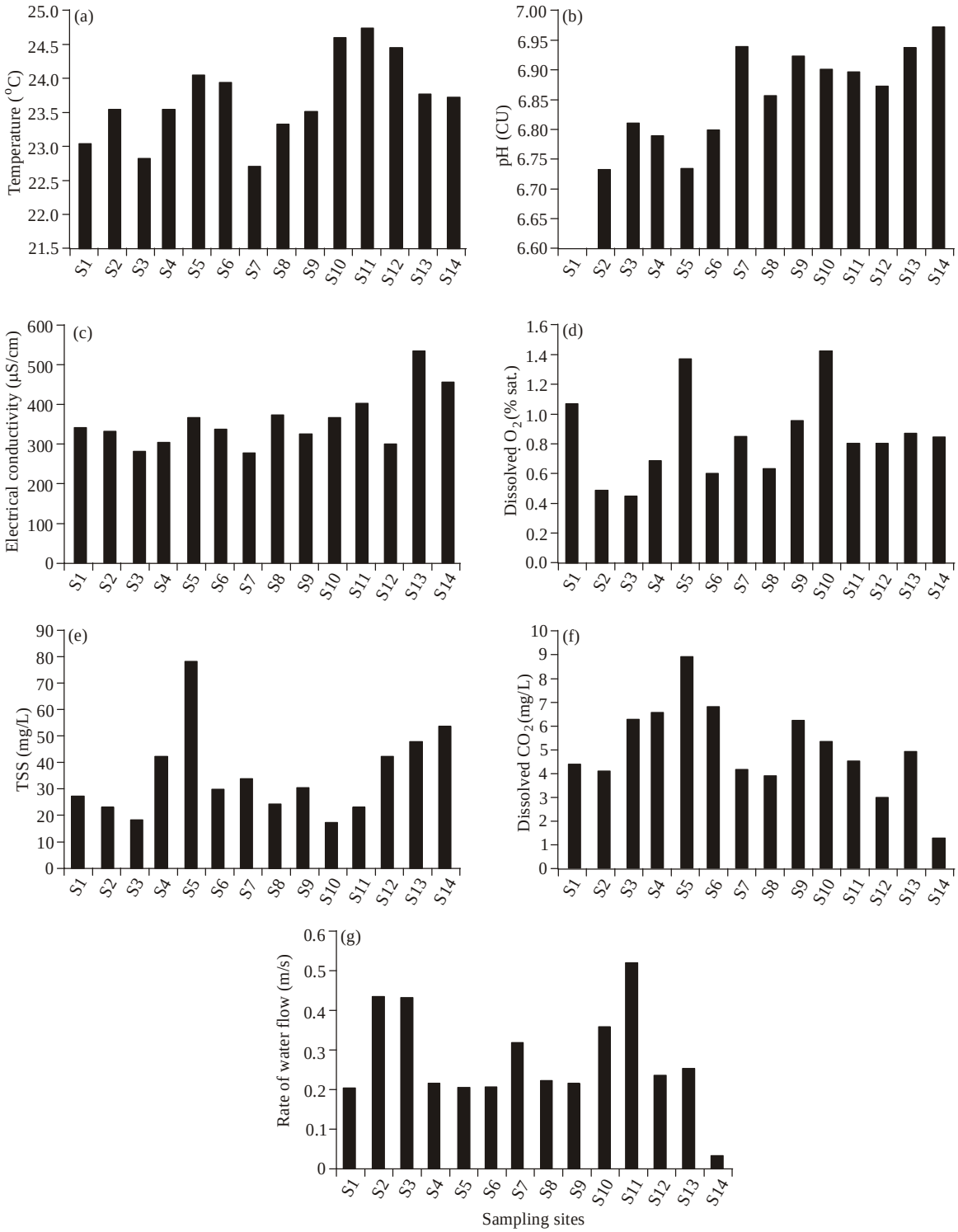

Fig. 2: Average values of physico-chemical parameters of surface waters: (a) Temperature, (b) pH, (c) Electrical conductivity, (d) Dissolved oxygen, (e) Total suspended solids, (f) Dissolved carbon dioxide, (g) Rate of water flow

total suspended solids and the water temperature respectively.

To measure the rate of water flow, a stop watch was used. The distance that the indicator of water

Table 1: Average and dispersion variables for physico-chemical characteristics of water samples

Parameter	Max	Min	Avg.	S.D.*
pH (UC)	7.70	5.21	6.90	0.28
Electrical conductivity (µs/cm)	662.00	3.70	348.00	140.60
Dissolved O_2 (mg/L)	3.40	0.20	0.84	0.70
Dissolved CO_2 (mg/L)	18.80	0.00	5.00	4.25
Total suspended solids (mg/L)	147.00	5.00	36.14	26.87
Rate of water flow (m/s)	0.55	0.02	0.30	0.13
Temperature (°C)	25.50	22.00	23.70	0.84

Max: Maximum; Min: Minimum; Avg.: Average; *S.D.: Standard deviation

movement would move though in all locations was 7 m upstream-downstream. The techniques used in this physico-chemical part of the study are those recommended by Clesceri and Franson (1998). Figure 1 locates the different regions of the country where cases of cholera are documented (A) and the sites of specimen collection (B) in Yaoundé (the Center Region).

For bacteriological analyses, water samples collected in 500 mL sterile glass bottles were transported in refrigerated containers to the laboratory for analyses.

The cultures were performed on the culture media as recommended by BioMérieux for the growth and isolation of *Vibrio* species. Specimen enrichment was carried out by suspending 5 mL of the specimen in 10 mL of the culture broth. The mixture was incubated aerobically for 24 h at 37°C.

From the resulting microbial inoculums, subcultures were performed on Thiosulfate Citrate Bile Salt (TCBS) agar and re-incubated aerobically at 37°C overnight.

At the end of the incubation period, suspected bacterial colonies were either yellow (that is positive, which implies that bacteria used saccharose) or green (that is negative, implying that bacteria did not use saccharose). From these colonies, subcultures were once again performed on alkaline nutrient agar and re-incubated aerobically at 37°C overnight. Upon revelation, translucent colonies on nutrient agar were targeted. Characteristic polar mobility was assessed in wet preparation under light microscopy at high power field (40x). Suspected organisms were Gram negative bacteria on Gram stain at 100x objective (either curved or not) and positive for oxidase. Other biochemical tests of identification were run according to the protocols recommended in classical bacteriology (Rémic, 2007).

The polyvalent O1 anti serum was used to identify isolates of O1 antigen positive *Vibrio cholerae*. Spearman correlation test was used to assess the strength of association between the presence of *Vibrio* and physico-chemical characteristics of the water; the mean differences were assessed with the Z score. The significance level used was 5%.

RESULTS

Physico-chemical characteristics: Data on the environmental determinants targeted were recorded and analyzed; Physico-chemical characteristics are recorded in Fig. 2. Table 1 summarizes the maximal, minimal, average values and fluctuations of the environmental factors investigated.

The average pH values fluctuated, but remained relatively low with a range of 2.49 CU (Table 1) along the study. The highest values were recorded in February and March (during the dry season) and the lowest in June (during the rainy season). No significant variations were observed.

Average values for electrical conductivity varied with time and space. The average range associated was 658.3 µS/cm (significant variations between many collection sites). Like the pH, their highest values were recorded during the dry season and their lowest during the rainy season.

Important variations in space and time were recorded for the values of dissolved gases. But, oxygen concentration (saturation percentage) fluctuated less than carbon dioxide. The standard deviations where respectively, equal to 0.7 and 4.25**.**

Concentration in total suspended solids also varied significantly (5 to 147 mg/L), unlike the rate of water flow and water temperature that ranged from 0.02 to 0.55 m/s and from 22 to 25.5°C, respectively.

The values of electrical conductivity and total suspended solids were the most dispersed around their mean values (standard deviation equal to 140.6 and 26.87, respectively) while those of the pH and the rate of water flow were most concentrated (standard deviation respectively equal to 0.28 and 0.13).

Bacteriological parameters:

Species richness and distribution: Microbiological analyses of specimens resulted in the identification of six *Vibrio* species: *V. vulnificus*, *V. fluvialis*, *V. mimicus*, *V. parahaemolyticus*, *V. alginolyticus* and *V. cholerae.* Members of these bacterial species appeared as green, yellow or green/yellow colonies on

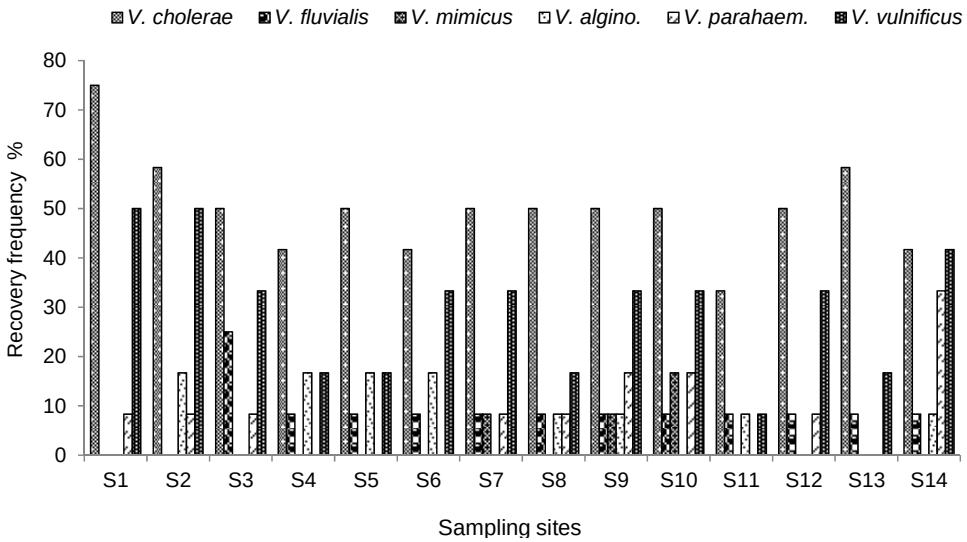

Fig. 3: Isolation frequencies of different species of *Vibrio* in surface waters in Yaoundé

Table 2: Spatial distribution of *Vibrio* species in Yaoundé

Subdivision and collection sites	Yaoundé 1			Yaoundé 5		Yaoundé 4	Yaoundé 6			Yaoundé 3				
Vibrio species	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14
V. cholerae	x	x	x	x	x	x	x	x	x	x	x	x	x	x
V. fluvialis			x	x	x	x	x	x	x	x	x	x	x	x
V. mimicus							x		x	x				
V. alginolyticus		x		x	x	x		x	x		x			x
V. parahaemolyticus	x	x	x				x	x	x	x		x		x
V. vulnificus	x	x	x	x	x	x	x	x	x	x	x	x	x	x

X: Presence of *Vibrio*

TCBS agar. They were further distinguished based on other characteristics like the ability to grow in salty environments or the presence of O1 antigen (O1 positive *V. cholerae*). Colonies of *V. alginolyticus*, *V. cholerae* and *V. fluvialis* were yellow, those of *V. parahaemolyticus* and *V. mimicus* were green, while *V. vulnificus* appeared as yellow/green on TCBS. All species grew at 3% of sodium chloride, while other salt concentrations were more selective. Strains of *V. cholerae* and *V. mimicus* grew in the absence of salt in water; while those of *V. alginolyticus* were shown to grow at the highest concentrations of sodium chloride (8%). Figure 3 and Table 2 display the spatial distribution the frequencies of recovery and species diversity recorded.

Strains of *V. cholerae* were isolated from all the collection sites (100%) targeted in the present study. The highest rate of isolation for this species (79%) was observed at S1 upstream of the Mfoundi River situated at Etoa-Meki. Isolates of *V. vulnificus* were the second most frequent and were obtained from 93% (13 out of 14) of sample collection sites. The highest frequencies were observed at S1, S2 and S14.

Station S9 situated on the Biyémé River at Biyem-Assi was the only site where all the six *Vibrio* species were isolated (highest species diversity). At this collection site, *V. cholera*, *V. vulnificus* and *V. parahaemolyticus* were the most frequent species, but the isolation frequency of *V. cholerae* was lower than reported at S1, S2. Isolation frequencies for *V. mimicus*, *V. fluvialis* and *V. alginolyticus* were similar. Species diversity was also documented in other collection sites namely S7, S8, S10, S14 and S2, S3, S4, S5, S6, S11, S12 where five and four species were isolated, respectively. The lowest diversities were obtained at S1 and S13 with three species.

Overall, isolates from other *Vibrio* species were recovered in various but lower frequencies than *V. cholera*e and *V. vulnificus*.

The strength of association between the presence of these organisms and environmental determinants was assessed with Spearman correlation test. The results obtained are presented in Table 3. Collectively, significant positive correlation was observed between the isolation frequencies of *V. mimicus* and dissolved oxygen, while *V. parahaemolyticus* isolation rates showed a significant decrease with increased dissolved carbon dioxide concentration ($p<0.05$).

Serological identification: A total of 50 isolates of *V. cholerae* were tested for the presence of the O1 antigen

Table 3: Test of association between physico-chemical characteristics and the presence of isolates from various *Vibrio* species

Vibrio species	T°	pH	E.C	TSS	O_2	CO_2	RWF
V. cholerae	-0.443	-0.459	0.021	-0.064	0.201	-0.038	-0.108
V. fluvialis	-0.168	0.271	-0.193	-0.093	-0.267	0.234	0.203
V. mimicus	0.127	0.393	-0.174	-0.318	0.541*	0.054	0.151
V. alginolyticus	0.151	-0.397	-0.052	0.303	-0.197	0.399	-0.136
V. parahaemolyticus	-0.090	0.452	0.108	-0.059	0.135	-0.617*	-0.404
V. vulnificus	-0.356	-0.243	-0.274	-0.263	-0.077	-0.357	-0.154

$p<0.05$; T°: Temperature; E.C: Electrical conductivity; TSS: Total suspended solids; O_2: Dissolved O_2; CO_2: Dissolved CO_2; RWF: Rate of water flow

known to occur in most epidemic strains. The slide agglutination assay resulted in positive reactions on 8 out of 50 of isolates (16%).

DISCUSSION

The present study reports isolation of six pathogenic species of *Vibrio* in water resources used by human populations in Yaoundé. All settings were contaminated. *Vibrio cholerae*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* were commonly isolated.

Of the *Vibrio* that have any clinical significance to humans, *V. cholerae*, the agent of cholera, is the most important (Fournier, 1998). *V. parahaemolyticus* has long been recognized as a causative agent of gastroenteritis and the most common non-cholera *Vibrio* species reported to cause infection related deaths (Chowdhury *et al*., 1990). Strains of this species are also incriminated in wound infections but less commonly than in seafood borne diseases. *V. alginolyticus* is medically important since it causes otitis and wound infections (Fournier, 1998). *V. fluvialis* has been incriminated in sporadic infections and outbreaks of diarrhea in humans (Huq *et al*., 2005). The pathogenicity of clinical isolates of *V. mimicus* with freshwater prawns has been described (Chowdhury *et al*., 1990). *V. vulnificus* is an emerging pathogen of humans causing wound infections, gastroenteritis or syndrome known as primary septicemia (Todar, 2012).

The results of the present works are consistent with the previous findings about poor sanitation and potential health risk (Njiné *et al*., 2001; Nguendo Yongsi *et al*., 2008; Fotsing Kwetché *et al*., 2008; Tamatcho Kweyang *et al*., 2009), suggesting the likely origin of the first cholera outbreak ever documented in Yaoundé. The most exposed location to *V. cholerae* was Etoa-Meki upstream the Mfoundi River where most contaminated resources are Situated (S1). The highest rate of isolation of *V. cholerae* at S1 was associated with the lowest species diversity. The species diversity varied but was relatively high (4 to 6 species) in twelve settings out of the fourteen investigated. This species diversity highlights the fact that growth conditions (physico-chemical conditions) are suitable for a large variety of microorganisms.

The range of temperature observed here (22.6 to 24.8°C), common in tropical areas is conducive to bacterial growth (Dumontet *et al*., 2000; David *et al*., 2005). Probably, the temperature played a crucial role in the bacterial growth observed in all settings investigated. Its values did not vary much from one site to the other (standard deviation 0.84).

Data analyses also demonstrated that in the average, surface water resources are generally neutral or slightly acidic. Because *Vibrio* strains are recovered in these conditions, some questions may arise about the ability of these bacteria to survive in diverse environmental conditions. In fact, it is recommended to use alkaline culture media for their growth in laboratories (Collins, 2003). No significant associations were observed between the pH and the rate of isolation of different *Vibrio* species. Because bacterial isolation was also observed in sample with lower pH and, assuming that the most frequently isolated species were those whose environmental conditions were most favorable for growth and spread, strains of *V. cholerae* (and not those from other species) probably adapted better to lower pH. This pH-dependent growth may explain both why strains of this species are found in almost all settings in varying frequencies (the range of pH that allows their growth may be broader than currently believed). The low standard deviation associated with the pH demonstrates that, like the temperature, it could not influence any geographical distribution of strains within the Yaoundé city areas, making therefore all settings equally vulnerable.

The average values of electrical conductivity are high. This is in accordance with reports from other investigators that water resources in Yaoundé contain higher concentrations of minerals including iron, calcium, magnesium and aluminum (Yongue-Fouateu, 1986; Suchel, 1988; Nola *et al*., 1998) compared to the standard recommended in Rodier (1996). Time and space variations in their average values are possibly associated with human activities including agricultural practices using artificial fertilizers, husbandries and traditional slaughtering houses by stream sides that are common in Yaoundé. Other potential sources of

minerals are brewery wastes which are poured without adequate treatment into water streams. The direct impact of these activities on the quality of water and the life of aquatic organisms had clearly been described in other settings (Seidu *et al*., 2008). In fact, minerals are important factors in bacterial growth. Because outbreaks are mostly documented during dry seasons (Chowdhury *et al*., 1989) when the highest values of pH and electrical conductivity are recorded, isolation rates in the present study suggest that they are favorable to *Vibrio* growth and dissemination. This is consistent with other reports (Huq *et al*., 2005) that environmental factors that positively correlate the recovery rates of *Vibrio* isolates in water included temperature, electrical conductivity and decreased rainfall.

The variation of dissolved carbon dioxide and oxygen concentrations may be favored by activities of aerobic microorganisms on organic matters that are permanently provided by human activities. Except for *V. mimicus* that was positively associated with water content in dissolved oxygen ($p<0.05$), no significant associations were observed with this gas. *V. parahaemolyticus* was positively associated with dissolved carbon dioxide ($p<0.05$).

Concerning the total suspended solids, their concentrations were elevated in the resources investigated compared to the norms (Rodier, 1996). These concentrations are in agreement with the above development about permanent provision of materials by human activities and might explain, at least in part, the high concentrations of CO_2 that eventually make water resources more acidic.

The most contaminated samples were observed at S1, situated at Etoa-Meki upstream the Mfoundi River. It is a swampy and the dirtiest area along the river.

Slide agglutination tests resulted in the detection of O1-antigen-positive strains of *V. cholerae*. Also, all the species identified in the present investigation are potential human pathogens. In addition, it is increasingly recognized that strains of *V. cholerae* that are etiologies of cholera epidemics are lysogenized strains, not necessarily only those belonging to the O1 and O139 serogroups (Dalsgaard *et al*., 2001). Therefore, population exposure might be higher than currently estimated in Yaoundé. Although environmental conditions that enable optimum replication and dissemination of the filamentous bacteriophage from which the toxin genes are transferred to *V. cholerae* have not yet been fully investigated thorough, it is likely that they have only recently been met in Yaoundé aquatic environment. These developments highlight the limitation associated with O1 antigen based identification techniques commonly used to identify pathogenic strains of *V. cholerae* in epidemiological surveys. This limitation may explain the large number of non-elucidated diarrheal cases in endemic areas and the necessity to manage surface water for a safer life in Yaoundé.

CONCLUSION

Six potential disease causing *Vibrio* species were isolated during this investigation conducted in the rivers of Yaoundé. Environmental determinants like electrical conductivity and temperature appear to be important in the growth and dissemination of these bacteria. Because resources from which they are frequently isolated are also those that are commonly used by the local populations in their daily activity, their presence appears to be a serious potential threat for public health. The present results suggest the need for surface water resources management and for the detection of *Vibrio* species other than *V. cholerae* as the routine process in clinical and research laboratories for efficient disease prevention and management.

ACKNOWLEDGEMENT

Professor Patrick Grimont (Institut Pasteur in Paris) advised, oriented and revised the research protocol. We are very grateful for his invaluable contribution. We are also highly indebted to Dr Marie-Christine Fonkoua (Centre Pasteur du Cameroon) who provided us with the O1 anti serum used in the detection of O1 positive *V. cholera* strains.

REFERENCES

Chowdhury, M.A.R., A.H. Yamanaka, S. Miyoshi, K.M.S. Aziz and S. Shinoda, 1989. Ecology of *Vibrio mimicus* in aquatic environments. Appl. Environ. Microbiol., 55: 2073-2078.

Chowdhury, M.A.R., A.H. Yamanaka, S. Miyoshi and S. Shinoda, 1990. Ecology and seasonal distribution of *Vibrio parahaemolyticus* in aquatic environments of a temperate region. FEMS Microbiol. Ecol., 74: 1-10.

Clesceri, L.S. and M.A.H. Franson, 1998. Standard Methods for the Examination of Water and Wastewater. 20th Edn., American Public Health Association, Washington DC, pp: 1220.

Collins, A.E., 2003.Vulnerability to coastal cholera ecology. Soc. Sci. Med., 57: 1397-1407.

Dalsgaard, A., O. Serichantalers, A. Forslund, W. Lin, J. Mekalanos and E. Mintz, 2001. Clinical and environmental isolates of *Vibrio cholerae* Serogroup O141 Carry the CTX phage and the genes encoding the toxin-coregulated pili. J. Clin. Microbiol., 39(11): 4086-4092.

David, E.J. and B.R. Joan, 2005. Review of factors affecting microbial survival in ground water. Environ. Sci. Technol., 39(19): 7345-7365.

Dosso, M., M. Coulibaly and A. Kadio, 1998. Place of bacterial diarrhea in developing countries. Bulletin de la Société de Pathologie Exotique, 91(5BIS): 397-449, (In French).

Dumontet, S., K. Krovacek, B.S. Svenson, V. Pasquale, B.S. Baloda, *et al*., 2000. Prevalence and diversity of *Aeromonas* and *Vibrio* spp. in coastal waters of southern Italy. Comp. Immun. Microbiol. Infect. Dis., 23: 53-72.

Duraku, E. and A. Panariti, 1998. A case of acute gastroenteritis caused by *Vibrio cholerae* non O1. Rev. Inter. Serv. Santé des Forces Armées. 71(10-12): 298-300, (In French).

Fotsing Kwetché, P.R., J. Kamgno, F. Mouchet, A. Banza, P. Sorlin and J. Thonnon, 2008. Enteroaggregative *Escherichia coli*: A public health hazard in Yaoundé, Cameroon? Int. J. Biol. Chem. Sci., 2(3): 272-280.

Fotsing Kwetché, P.R., B.P. Tamatcho Kweyang, T. Njiné and M. Nola, 2010. *Vibrio cholerae* in watery environment in Yaoundé: A public health hazard? Book of abstracts. 17th Annual Conference of Cameroon Bioscience Society (CBS), pp: 34.

Fournier, J.M., 1998. Choléra. Encycl. Med. Chir. (Elsevier, Paris), Maladies infectieuses. 8-028-F-10, p: 5.

Fournier, J.M. and M.L. Quilici, 2002. Cholera vibrio infections not. Encycl. Med. Chir., (Editions scientifiques et médicales Elsevier, Paris), Maladies infectieuses. 8-026-F-15.

Guévart, E., J. Noeske, J. Solle, J.M. Essomba and E. Mbonji, 2006. Determinants of cholera in Douala. Méd. Trop., 66: 283-291.

Huq, A., R.B. Sack, A. Nizam, I.M. Longini, G.B. Nair, *et al*., 2005. Critical factors influencing the occurrence of *Vibrio cholerae* in the environment of Bangladesh. Appl. Environ. Microbiol., 71(8): 4645-4654.

Leclerc, H., 2003. Are there opportunistic bacterial infections transmitted by water supply? J. Eur. Hydrol., 34(1): 11-14.

Nguendo Yongsi, H.B., G. Salem and J.P. Thouez, 2008. Health risks associated with methods of excreta sanitation in Yaounde. Cameroon. N.S.S., 16: 3-12.

Njiné, T., A. Monkiédjé, M. Nola and V. Sikati Foko, 2001. Assessment of pollution load and the bacterial load of releases of activated sludge processes in Yaounde (Cameroon). Cahiers Santé., 11: 79-84, (In French).

Nola, M., T. Njiné and C. Boutin, 1998. Variability of groundwater quality in a few stations in Yaoundé (Cameroon). Mémoires de Biospéologie, 25: 183-191, (In French).

Rémic, 2007. Repository in Medical Microbiology (Bacteriology and Mycology). 3rd Edn., SFM, Paris. Vivactis Plus, Paris, France, pp: 232.

Rodier, J., 1996. Water Analysis. 8th Edn., Dunod, Paris, France, pp: 1384.

Seidu, R., A. Heistad, P. Amoah, P. Drechsel, P.D. Jenssen and T.A. StenstrÖm, 2008. Quantification of the health risk associated with wastewater reuse in Accra, Ghana: A contribution towards local guidelines. J. Water Health, 6(4): 461-471.

Suchel, B., 1988. Rainfall patterns and rainfall regions in Cameroon. Travaux et documents de géographie tropicale (C-E-CNRS), 5: 1-288.

Tamatcho Kweyang, B.P., P.R. Fotsing Kwetché, T. Njiné, M. Nola and P. Djogo, 2009. Some factors determining the distribution of *Vibrio* species in the aquatic environment of Yaounde, Cameroon. Cameroon J. Exp. Biol., 5(2): 96-103.

Tamatcho Kweyang, B.P., P.R. Fotsing Kwetché, T. Njiné and M. Nola, 2010. Pathogenic *Vibrio* species in tropical waters: Case in Yaoundé a contribution towards disease prevention. Book of abstracts 17th Annual Conference of Cameroon Bioscience Society (CBS), pp: 32.

Todar, K., 2012. Online Textbook of Bacteriology. Retrieved from: http:// text book of bacteriology. net/index.html.

WHO, 2004. Guidelines for Drinking Water Quality: Recommendations. 3rd Edn., World Health Organization, Geneva, ISBN: 9241546387, pp: 515.

Yongue-Fouateu, R., 1986. Contribution to the petrographic study of alteration and encrustation ferruginous facies migmatitic gneisses of the Yaoundé area. Ph.D. Thesis, Université de Yaoundé, (In French).